R-4739-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Moutsatsos et al..	Examiner:	Sandra W.
Serial No.:	09/148,234	Group Art Unit:	1636
Filed:	September 4, 1998		
Title:	GENETICALLY ENGINEERED CELLS WHICH EXPRESS BONE MORPHOGENIC PROTEINS		

DECLARATION UNDER RULE 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, DC 20231

I, Dan Gazit, a citizen of Israel, residing at 46 Perez Berenstein Street, Jerusalem, 96920, hereby declare:

1. I am a Professor and Graduate Program Director of the Hebrew University-Hadassah Medical Center. I have a Ph.D. in bone biology from the Hebrew University, Jerusalem Israel. My field of expertise is skeletal biotechnology and developmental molecular biology. Specifically, I have been involved in the study of Adult Human Mesenchymal stem cells and Skeletal tissue engineering.

2. My Curriculum Vitae and list of publications are attached herewith as Appendix A.

3. I have reviewed the subject Application and the Office Action dated February 7, 2002 issued by the United States Patent and Trademark Office in connection with the subject Application. The subject Application describes *inter alia ex-vivo* methods of transforming bone progenitor cells, including mesenchymal stem cells with a nucleic acid, which encodes for BMP2 protein, for the implantation in a subject in need for bone repair or regeneration.

4. Claim 11 of the subject Application recites a method for producing cells for implantation at the site of a bone infirmity in a human, said method comprising the steps of:

   (a) transforming a cultured human progenitor cell or a bone marrow stromal cell with a DNA encoding bone morphogenesis protein 2 (BMP-2); and

   (b) culturing the cultured human progenitor cell or bone marrow stromal cell transformed in step (a);

   whereby cells are produced for implantation at the site of a bone infirmity in a human.

5. In the Office Action, the Examiner rejected Claim 11 of the above-identified Application as allegedly being obvious to one skilled in the art, based on United States Patent No. 5,763,416 (Bonadio et al.). The Examiner asserted that it would have been obvious to one of ordinary skill in the art at the time of filing the Application to obtain Applicants' invention, namely a method for producing cells for implantation at the site of a bone infirmity in a human, comprising the steps of: (i) transforming a cultured human progenitor cell or a bone marrow stromal cell with a DNA encoding bone morphogenesis protein 2 (BMP-2); and (ii) culturing the cultured human progenitor cell or bone marrow stromal cell transformed in step (i); whereby cells are produced for implantation at the site of a bone infirmity in a human. Specifically, the Examiner asserted that since Bonadio allegedly discloses "a method of producing cultured or bone marrow stromal cells for implantation at the site of bone infirmity by transforming the cells with recombinant bone morphogenetic protein, a person of ordinary skilled in the art would have had a reasonable expectation of success in the producing the instant claimed invention given the teachings of US Pat No. 5,763,416 or WO 96/39431 with US Pat. No. 5,645,084 and US Pat. No. 5,700,774".

6. The Examiner stated that Bonadio discloses direct gene transfer into regenerating bone in vivo by using bacterial galactosidase and insect luciferase (Example VIII), adenoviral gene transfer into regenerating bones in vivo (Example IX), transfer of osteogenic gene stimulated bone regeneration/ repair

in vivo (Example X), transfer of genes to Achilles tendon and to cruciate ligament in vivo (Example XI).

7. The Examiner is incorrect in his assertion. It is my opinion that direct *in-vivo* gene transfer is not predictive *a priori* for use of *ex-vivo* engineered cells for implantation for bone reapir, and therefore obtaining credible and/or functional results is not *a priori* an expected and/or obvious outcome. Specifically, it would not have been obvious that a person of ordinary skill in the art would have had a reasonable expectation of success in the producing of the instant claimed invention given the teachings of Bonadio in combination with He, McKay and/or Hattersley. Based on Bonadio, one skilled in the art would not have been able to predict *a priori* based on a direct *in-vivo* gene transfer approach disclosed in Bonadio that *ex-vivo* methods of transforming mesenchymal stem cells with a nucleic acid which encodes for BMP2 protein, would be effective for implantation in a subject in need for bone repair or regeneration. Further, there is no credible evidence in Bonadio that one could make *ex-vivo* engineered mesenchymal stem cells with a nucleic acid which encodes for BMP2 protein, that upon implantation is effective for bone repair or regeneration.

8. In addition, it was unexpected that *ex-vivo* therapy, which involves both autocrine and paracrine mechanisms would achieve a higher efficiency of bone regeneration in comparison to the described *in-vivo* therapy of Bonadio.

9. Bonadio does not provide any scientific data and/or experiments directed to demonstrate *ex-vivo* methods of transforming mesenchymal stem cells with a nucleic acid, which encodes for BMP2 protein, for implantation in a subject in need of bone repair or regeneration. Moreover, Bonadio does not disclose *ex-vivo* methods of transforming mesenchymal stem cells with a nucleic acid, which encodes for BMP2 protein, for the implantation in a subject in need of bone repair or regeneration. Bonadio instead describes direct *in-vivo* gene therapy, which involve only autocrine mechanisms of action.

10. In the above-identified Application, Applicants have demonstrated that an unexpected higher efficacy of the extent of bone regeneration is obtained via *ex-vivo* engineered cell implantation methods for bone regeneration due to the participatory effects mediated by autocrine (effect on the cells that are to be implanted) and paracrine (effect on the host mesenchymal cells) mechanisms involved (See Specification, Example 8, page 13).

11. In a study conducted which was published in *Gazit et al., 1999, J Gene Med 1: 121-133,* a copy of which is attached hereto as Appendix B, the biologic and functional effect of *ex-vivo* engineered progenitor cells (C3H-BMP2) (10^6, expressing 5 ± 2.3 ng/24 hours/10^7 cells) administered to a subject, was compared to the direct administration of 3 μg recombinant human BMP2. The results demonstrated that the direct introduction of the protein did increase the formation of bone tissue, but resulted in disorganized formation of bone, i.e. the *de novo* bone formation was not functional because the bone formation was not in alignment with the original defect edge. Thus, the bone regeneration induced by *ex-vivo* genetically engineered progenitors expressing BMP-2 succeeded in functional bone formation where that of direct protein delivery did not. The results of the direct administration of recombinant human BMP2 demonstrated that it is unpredictable to obtain credible functional bone formation results based on direct *in-vivo* experiments.

12. Further, the healing effect of engineered progenitor cells (C3H-BMP2) was compared with the effect on engineered non-progenitor cells (CHO-BMP2). Unexpectedly, the results showed that the effect on bone regeneration at eight weeks after transplantation was 1.65 fold greater in C3H-BMP2 transplants in comparison to the CHO-BMP2 transplants. In addition, the bone formation in CHO-BMP2 was impaired and disorganized in comparison to the C3H-BMP2 cells. This was more surprising considering the fact that the *in-vitro* secretion of recombinant human BMP2 detected for C3H BMP2 was 168 times less than the amount secreted from CHO BMP2 (which secretes 841 ± 88 ng/24 hours/10^7 cells). Thus, it was concluded that successful functional bone formation repair achieved by engineered C3H-BMP2 cells required an effect of both autocrine and

paracrine mechanisms, as opposed to the effect achieved by the CHO-BMP2 cells, which involved solely a paracrine mechanism.

13. In a study conducted which was published in Moutsatsos et al. 2001, Molecular Therapy 3:449-461, a copy of which is attached hereto as Appendix C, it was also quantitatively demonstrated that the implantation of *ex-vivo* engineered MSCs yielded more bone tissue in segmental defects than direct administration of rhBMP-2 protein.

14. Thus, the above experiments demonstrate that transplanted C3H-BMP2 exhibit greater efficacy both quantitatively and qualitatively than recombinant human BMP2 administration, or administration of CHO-BMP2 cells. The superior effect was attributed to the combined involvement of both autocrine and paracrine mechanisms in mesenchymal stem cell-based *ex-vivo* gene therapy which would not have been expected, *a priori*, based on a direct *in-vivo* approach such as in the Bonadio disclosure. Further, based on the above experiments there is no credible evidence in Bonadio that one could make *ex-vivo* engineered mesenchymal stem cells with a nucleic acid which encodes for BMP2 protein, that upon implantation is effective for bone repair or regeneration.

15. Therefore, in view of the reasons and the facts described above, one skilled in the art would not be able to predict *a priori* that a person of ordinary skill in the art would have had a reasonable expectation of success in producing the instant claimed invention given the teachings of Bonadio.

The undersigned further declares that all statements made herein of his own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: March 6, 2003

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